DNA nanotechnology for molecularly self-assembled nanoparticles and their drug delivery applications

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ABSTRACT SUMMARY
Nanoparticles are useful for delivering therapeutics into cells¹². However, size, shape, surface chemistry and the presentation of targeting ligands on the surface of nanoparticles can affect circulation half-life and biodistribution, cell specific internalization, excretion, toxicity, and efficacy³⁷. A variety of materials have been explored for delivering small interfering RNAs (siRNAs) - a therapeutic agent that suppresses the expression of targeted genes⁸. However, conventional delivery nanoparticles such as liposomes and polymeric systems are heterogeneous in size, composition and surface chemistry, and this can lead to suboptimal performance, lack of tissue specificity and potential toxicity¹⁰¹². Here, we show that self-assembled DNA tetrahedral nanoparticles with a well-defined size can deliver siRNAs into cells and silence target genes in tumours.

INTRODUCTION
Monodisperse nanoparticles are prepared through the self-assembly of complementary DNA strands. Because the DNA strands are easily programmable, the size of the nanoparticles and the spatial orientation and density of cancer targeting ligands (such as peptides and folate) on the nanoparticle surface can be precisely controlled. We show that at least three folate molecules per nanoparticle is required for optimal delivery of the siRNAs into cells and, gene silencing occurs only when the ligands are in the appropriate spatial orientation.

EXPERIMENTAL METHODS
Preparation of self-assembled DNA/siRNA nanoparticles (ONPs). Six single stranded DNAs (IDT) and six double-stranded siRNAs (Alnylam) were annealed to prepare ONPs. DNA strands (final strand concentration, 3.3 mM each) were mixed in an equal molar ratio in 1X PBS and a six-fold molar excess of siRNA strands was added to the solution. The solution was heated to 90 °C for 2 min and rapidly cooled to 4 °C to generate ONPs (particle concentration, 3.3 μM).

In vitro testing of ONPs. HeLa cells, modified to stably express both firefly and Renilla luciferase genes, were used for in vitro screening of the ONPs. ONPs with different ligands were applied to 1.5 × 10⁶ HeLa cells in medium containing serum. Firefly luciferase silencing was assessed 24 h post-transfection using a Dual-Glo Luciferase Assay kit (Promega). GFP-KB cells were also used for in vitro screening.

siRNA delivery in mice using ONPs. All animal experiments were conducted using institutionally approved protocols. Luc-KB tumour-bearing female BALB/c nude mice (Charles River Laboratories) received tail-vein or intratumoral injections of either PBS (negative control) or ONPs containing anti-Luc siRNA diluted in PBS (n = 7 for each group; siRNA concentration, 2.5 mg/kg). Two days post-injection, bioluminescence intensity (BLI) in KB tumours was measured using an IVIS Lumina imaging system.

RESULTS AND DISCUSSION
To ascertain whether ONPs can provide effective targeted delivery of siRNA to human cancer cells, we conjugated various cancer targeting ligands from peptides to small molecules. Among the 28 different targeting ligands tested here, folic acid (FA)-conjugated ONPs exhibited the greatest gene silencing, with 50% reduction of firefly luciferase expression in HeLa cells in a dose-dependent manner (Fig. 1).

Figure 1. Screening of tumour-targeting ligands using ONPs in a luciferase silencing assay in HeLa cells (control (ONPs without targeting ligands); LF (lipofectamine RNAiMax); various cationic peptides.)
Figure 2. In vitro and in vivo gene silencing using ONPs

(a) Structure and orientation of the ligand (bullet shapes on ends of siRNA strands). (b) GFP gene silencing efficiency varies with FA density on ONPs (n=4; siRNA: 35 nM). (c) Efficiency of gene silencing for ONPs (n=4; siRNA concentration, 35 nM; set A, FA on 1, 2 and 3a; set B, FA on 1, 2 and 3b; set C, FA on 1, 2 and 3c). (d) In vivo luciferase silencing in KB tumour xenografts (n=7; BLI, bioluminescence intensity; control, PBS injection; IT, intratumoral; IV, intravenous; ONPLuc, ONPs with folate-conjugated anti-luciferase siRNA; siLuc, folate-conjugated anti-luciferase siRNA). siRNA concentration, 2.5 mg/kg.

By using both FA-conjugated and non-conjugated siRNAs, the level of GFP gene silencing was investigated with various numbers of folate ligands (Fig. 2a), while maintaining the same number of siRNAs on each nanoparticle (Fig. 2b). Our results indicate that minimum of three folate ligands are required to achieve GFP gene silencing. Importantly, the orientation/location of the ligands dramatically affects gene silencing (Fig. 2c). When three FAs were decorated on the tetrahedron so that the local density was maximized (three folates encompass a face or vertex of the tetrahedron in Fig. 2a), GFP silencing was observed.

To assess the therapeutic potential of ONPs as cationic-free gene delivery carriers, we conducted in vivo gene silencing of firefly luciferase expressing KB xenografts. FA-conjugated ONPs with anti-luciferase siRNA were administrated at a dose of 2.5 mg kg⁻¹ of siRNA into mice, either by tail-vein injection or intra-tumour injection. Silencing was evaluated 48 h post-injection by measuring bioluminescent intensity in the tumour (Fig. 2d). Both tail-vein and intra-tumour injections resulted in a decrease of ~60% in bioluminescent intensity. When mice were injected by either mode of administration with FA-conjugated anti-luciferase siRNAs (not assembled into nanoparticles), no decrease in bioluminescent intensity was observed.

CONCLUSION

This study has demonstrated that six single-stranded DNA fragments, and six double-stranded siRNAs, can self-assemble in a one-step reaction to generate DNA/siRNA tetrahedral nanoparticles for targeted in vivo delivery. The overhang design of the DNA strands allows specific hybridization of complementary siRNA sequences and provides full control over the spatial orientation of the siRNA and the locations and density of cancer-targeting ligands. The ONPs can be modified with different tumour-targeting ligands by simple conjugation chemistry, extending the use of these nanoparticles to the treatment of various cancers.

REFERENCES


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